

Immunofluorescent staining for FLAG sequence in Affibody

Materials:

PBS

Blocking solution (1% BSA in PBS)

PFA 4% in PBS

Ice-cold Methanol

NucBlue® LiveReadyProbes® reagent (Life Technologies)

Procedure:

1. Wash the cells with PBS
2. Fixation:
 - Opt. A) ice-cold methanol for 5 min (fixation and permeabilization)
 - Opt. B) PFA 4% in PBS for 10 min (fixation only)
3. Wash with PBS twice
4. Blocking: add 1% BSA in PBS, for 1h at RT.
5. Add primary Ab (mouse anti-FLAG M2, Sigma, 1:500 dilution) in blocking solution for 2h at RT+1h at 37°C.
6. Wash with PBS 3 times
7. Add secondary Ab (Alexa488 or Alexa546-conjugated anti-mouse, Life Technologies, 1:300 dilution) in blocking solution for 1h at RT.

Secondary Abs w/o the primary Ab were used as control.
8. Wash with PBS 3 times
9. Nuclear staining: add NucBlue® in PBS for 15 min.
10. Wash with PBS
11. Imaging using laser scanning confocal microscope